JNPDD
Journal of Natural Product for Degenerative Diseases
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Comparative Angiotensin-Converting Enzyme Inhibitory Activity of Solvent Fractions from *Piper betle* L., *Morinda citrifolia* L., and *Dracaena angustifolia* Roxb.

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Received: 07 July 2026 / Accepted: 14 August 2026

ABSTRACT: Hypertension is a major cardiovascular risk factor, and angiotensin-converting enzyme (ACE) inhibition remains a key strategy for its management. The present study aimed to compare the ACE inhibitory activity of solvent fractions obtained from *Piper betle* L. leaves, *Morinda citrifolia* L. fruits, and *Dracaena angustifolia* Roxb. leaves. Ethanolic extracts were fractionated by liquid–liquid partitioning using n-hexane, ethyl acetate, and water. Fraction yields were determined, and in vitro ACE inhibitory activity was evaluated at 100 µg/mL using the ACE Kit–WST assay. Statistical analysis was performed using Welch ANOVA followed by the Games–Howell post hoc test. The aqueous fractions consistently produced the highest yields (56.10–87.99%), whereas the ethyl acetate fractions yielded the lowest amounts (5.15–14.47%). ACE inhibitory activity varied markedly among fractions, ranging from 28.23 ± 1.91% to 95.88 ± 0.89%. The ethyl acetate fraction of *P. betle* exhibited the strongest ACE inhibitory activity (95.88 ± 0.89%) and showed no significant difference from captopril ($p = 0.103$). In contrast, the highest activities of *M. citrifolia* and *D. angustifolia* were observed in the ethyl acetate (51.35 ± 0.94%) and n-hexane (59.68 ± 3.30%) fractions, respectively. These findings demonstrate that solvent fractionation effectively enriches ACE-inhibitory constituents and identify the ethyl acetate fraction of *P. betle* as a promising source of natural ACE inhibitors for further antihypertensive drug development.

KEYWORDS: ACE inhibition; antihypertensive; *Piper betle*; *Morinda citrifolia*; *Dracaena angustifolia*.

1. INTRODUCTION

Hypertension is a multifactorial cardiovascular disorder characterized by persistently elevated arterial blood pressure arising from dysregulation of the physiological processes responsible for maintaining vascular homeostasis [1,2]. The development of hypertension is associated with multiple pathophysiological mechanisms, including endothelial dysfunction, oxidative stress, impaired renal sodium handling, increased sympathetic nervous system activity, and excessive activation of the renin–angiotensin–aldosterone system (RAAS) [3,4]. In addition to these biological mechanisms, several modifiable risk factors, such as excessive dietary sodium intake, obesity, physical inactivity, tobacco use, alcohol consumption, and chronic psychosocial stress, contribute significantly to the development and progression of hypertension [5]. The global burden of hypertension has increased substantially over recent decades, with an estimated 1.4 billion adults aged 30–79 years living with the condition worldwide in 2024, making it a leading contributor to premature mortality and cardiovascular morbidity worldwide [6].

Among the mechanisms involved in blood pressure regulation, the renin–angiotensin–aldosterone system (RAAS) is recognized as a central contributor to the development and progression of hypertension [7].

How to cite this article: Agus K., Ernawati S., M. Hanafi, Puspa D N L., Syamsudin A. Ethnobotanical Survey of Medicinal Plants Used for the Treatment of Hypertension in Gunung Sari, Bogor, West Java, Indonesia. Journal of Natural Product for Degenerative Diseases. 2026; 4(1): 1-8.

ISSN: 3031-6847

Within this system, angiotensin-converting enzyme (ACE), a zinc-dependent dipeptidyl carboxypeptidase, catalyzes the conversion of angiotensin I to angiotensin II, a potent vasoactive peptide that induces vasoconstriction, stimulates aldosterone secretion, enhances sodium retention, and promotes oxidative stress and vascular remodeling [3,8]. Consequently, excessive ACE activity contributes to increased peripheral vascular resistance and persistent elevation of blood pressure, making ACE a primary molecular target for antihypertensive therapy [9].

The inhibition of ACE activity has become a key therapeutic strategy for hypertension management, and ACE inhibitors remain among the first-line antihypertensive agents recommended in clinical guidelines [10]. However, the long-term use of synthetic ACE inhibitors may be associated with adverse effects, including dry cough, hypotension, dizziness, angioedema, and renal dysfunction [11]. Consequently, increasing attention has been directed toward the identification of natural ACE inhibitors from medicinal plants as potential alternatives for hypertension management [12].

Ethnobotanical knowledge, together with prior phytochemical and pharmacological evidence, provides a strong rationale for selecting these three species. *P. betle* has long been used traditionally for various ailments [13] and contains phenolic and flavonoid constituents, including catechin and quercetin [14]. Its ACE-inhibitory potential is supported by early in vitro evidence (Somanadhan et al. 1999). Recent computational studies have identified several bioactive constituents of *P. betle* with promising ACE-inhibitory potential, exhibiting favorable binding affinities, favorable drug-likeness properties, and acceptable predicted safety profiles, supporting its potential as a source of natural antihypertensive agents [15].

M. citrifolia (noni) is traditionally used for hypertension and cardiovascular support [16], backed by the strongest direct evidence among the three species: dose-dependent in vitro ACE inhibition [17,18] and a human case study linking noni juice to ACE inhibition and reduced blood pressure [19]. *D. angustifolia* is traditionally used for preeclampsia, a hypertensive disorder [20], and its extracts show antioxidant and cardioprotective effects [21], but its ACE-inhibitory activity has never been directly tested, representing the specific gap this study addresses. Together, these three species offer converging but unequally developed evidence for ACE-inhibitory potential. Since phytochemical distribution varies with solvent polarity, solvent fractionation was used to enrich each species' active constituents. This study therefore compared the ACE inhibitory activity of solvent fractions from *P. betle* L., *M. citrifolia* L., and *D. angustifolia* Roxb. as candidate natural antihypertensive agents.

2. MATERIALS AND METHODS

2.1. Materials

The materials used in this study included crude extracts and solvent fractions of *P. betle* L. leaves, *M. citrifolia* L. fruits, and *D. angustifolia* Roxb. leaves, ACE Kit–WST (Dojindo Laboratories, Kumamoto, Japan), captopril (Sigma-Aldrich, USA), technical-grade n-hexane and ethyl acetate, and distilled water. The equipment used included a rotary evaporator (BÜCHI Rotavapor R-214, BÜCHI Labortechnik, Switzerland), an analytical balance (Mettler Toledo, Greifensee, Switzerland), a drying oven (Memmert, Germany), a separatory funnel (Pyrex, USA), micropipettes (Eppendorf, Germany), 96-well microplates, and a Thermo Scientific Varioskan LUX Multimode Microplate Reader (Thermo Fisher Scientific, USA).

2.2. Methods

2.2.1. Collection and Identification of Plant Material

Plant samples used in this study were obtained from the Indonesian Research Institute for Spice and Medicinal Crops (BALITTRO), Bogor, Indonesia. Specimens of the three species, *P. betle* L., *M. citrifolia* L., and *D. angustifolia* Roxb., were subsequently identified at the Center for Plant Conservation and Botanical Gardens, National Research and Innovation Agency (BRIN), to confirm their botanical identity prior.

2.2.2. Extraction and Liquid–Liquid Fractionation

The dried samples of *P. betle* leaves, *M. citrifolia* fruits, and *D. angustifolia* leaves were cleaned, pulverized, and extracted by repeated maceration using 96% ethanol. Briefly, the powdered plant materials were macerated in five consecutive 24-h cycles with periodic agitation and solvent replacement. The combined filtrates were concentrated under reduced pressure using a rotary evaporator and subsequently dried to obtain

crude ethanolic extracts. The crude extracts were then subjected to liquid–liquid partitioning based on solvent polarity. Briefly, 1 g of each crude extract was suspended in 25 mL of distilled water and successively partitioned with 25 mL of *n*-hexane and ethyl acetate using a separatory funnel. Each partitioning step was repeated five times to obtain the *n*-hexane, ethyl acetate, and aqueous fractions. The fractions were concentrated under reduced pressure using a rotary evaporator (50 °C, 70 rpm) and subsequently dried in an oven at 50 °C to remove residual solvents. The fractionation yield (%) was calculated relative to the dry weight of the corresponding crude extract [13-15].

2.2.3. In Vitro ACE Inhibitory Assay

Preparation of Enzyme and Indicator Solutions

The enzyme solution was prepared by dissolving Enzyme B in 2 mL of distilled water, transferring 1.5 mL of the resulting solution to the Enzyme A vial, and gently mixing on ice, after which the solution was stored at −20 °C. The indicator solution was prepared by separately dissolving Enzyme C and the coenzyme reagent in 3 mL of distilled water, then mixing 2.8 mL of each solution with the indicator reagent under chilled conditions to obtain the working indicator solution, which was subsequently stored at −20 °C.

Preparation of Sample Solutions

The n-hexane, ethyl acetate, and aqueous fractions of *P. betle* leaves, *M. citrifolia* fruits, and *D. angustifolia* leaves, together with captopril, were individually prepared as 1,000 µg/mL stock solutions in distilled water and subsequently diluted to 100 µg/mL for the ACE inhibitory assay.

ACE Inhibitory Assay Procedure

The ACE inhibitory assay was performed in 96-well microplates using the ACE Kit-WST (Dojindo Laboratories), based on the colorimetric method developed by Lam et al. (2007, 2008) [22,23], in which 3-hydroxybutyryl-glycyl-glycyl-glycine (3HB-GGG) served as the substrate. ACE cleaves 3HB-GGG into glycyl-glycyl-glycine and 3-hydroxybutyric acid (3HB). The released 3HB is subsequently oxidized through an enzymatic coupling reaction, generating NADH that reduces WST-1 to a yellow-colored formazan product measurable at 450 nm. Therefore, the absorbance intensity is directly proportional to ACE activity, and inhibition of ACE results in reduced formazan formation and lower absorbance values.

For the sample wells, 20 µL of sample solution was mixed with 20 µL of substrate buffer containing 3HB-GGG and 20 µL of enzyme solution. In Blank 1 (positive control), the sample solution was replaced with 20 µL of distilled water, whereas in Blank 2 (reagent blank), the enzyme solution was replaced with distilled water. The reaction mixtures were incubated at 37 °C for 60 min, followed by the addition of 200 µL of indicator solution and further incubation at room temperature for 10 min. Absorbance was measured at 450 nm using a microplate reader. For colored fractions, sample blanks consisting of 20 µL of sample solution and 240 µL of distilled water were prepared, and their absorbance values were used to correct for background interference. All measurements were performed in triplicate, and the percentage inhibition of ACE activity was calculated using the following equation:

$$ACE\ inhibition\ (\%) = \frac{A_{Blank\ 1} - A_{Sample}}{A_{Blank\ 1} - A_{Blank\ 2}} \times 100$$

Where $A_{Blank\ 1}$ is the absorbance of the positive control (without inhibitor), $A_{Blank\ 2}$ is the absorbance of the reagent blank, and A_{Sample} is the absorbance of the tested sample [13-15].

2.3. Data Analysis

The ACE inhibitory activity data obtained from the solvent fractions were subjected to statistical analysis and expressed as mean ± standard deviation (SD) of three independent measurements. Normality was assessed using the Shapiro–Wilk test, while homogeneity of variances was evaluated using Levene's test. Differences among groups were analyzed using Welch's one-way analysis of variance (Welch ANOVA). When significant differences were detected, pairwise comparisons were performed using the Games–Howell post hoc test. Statistical analyses were conducted using IBM SPSS Statistics version 26.0, and differences were considered statistically significant at $p < 0.05$.

3. RESULTS

3.1. Fractionation Yield of Plant Extracts

Figure 1 shows the yields of solvent fractions obtained from *P. betle*, *M. citrifolia*, and *D. angustifolia*. The yields of the n-hexane, ethyl acetate, and aqueous fractions ranged from 9.26–29.43%, 5.15–14.47%, and 56.10–87.99%, respectively. The highest yield was observed for the aqueous fraction of *M. citrifolia* (87.99%), whereas the lowest yield was recorded for the ethyl acetate fraction of *M. citrifolia* (5.15%). Overall, a similar yield distribution pattern was observed among the three plant species, with the aqueous fractions consistently showing the highest yields and the ethyl acetate fractions the lowest. The predominance of the aqueous fractions indicates that water-soluble constituents constituted a major portion of the extracted materials. Differences in fraction yields may reflect variations in the distribution of phytochemicals according to their polarity during the partitioning process.

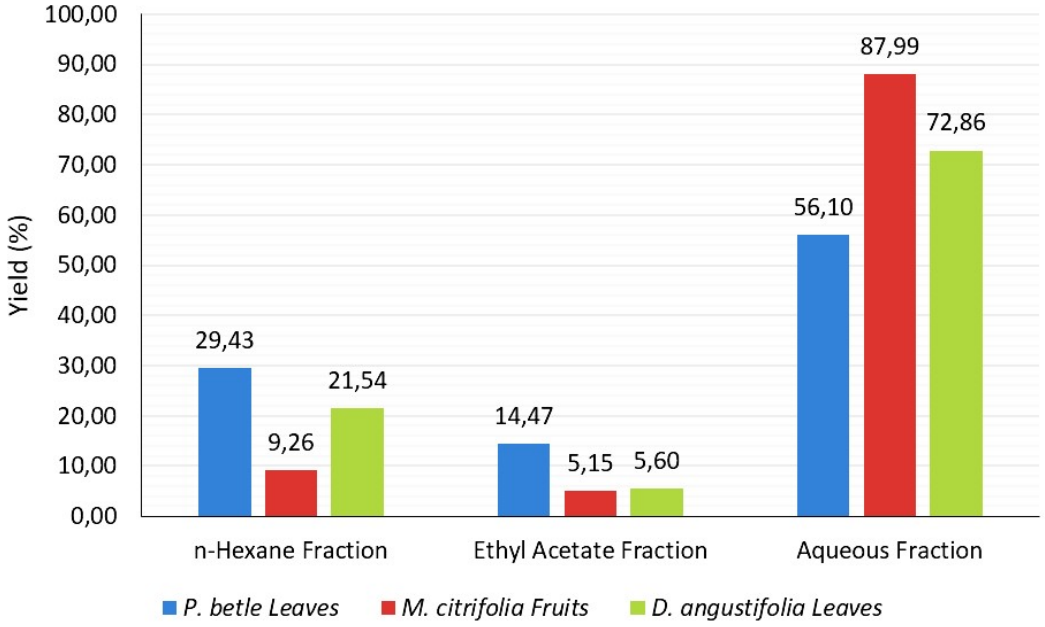

Figure 1. Comparison of the yields (%) of n-hexane, ethyl acetate, and aqueous fractions obtained from *P. betle* leaves, *M. citrifolia* fruits, and *D. angustifolia* leaves after liquid–liquid partitioning.

3.2. In Vitro ACE Inhibitory Activity

The ACE inhibitory activities of solvent fractions obtained from *P. betle* leaves, *M. citrifolia* fruits, and *D. angustifolia* leaves at a concentration of 100 µg/mL are presented in **Table 1**. ACE inhibitory activity varied markedly among the fractions, ranging from 28.23 ± 1.91% to 95.88 ± 0.89%. The ethyl acetate fraction of *P. betle* exhibited the strongest inhibitory activity (95.88 ± 0.89%), followed by its n-hexane fraction (68.75 ± 2.64%), whereas the aqueous fraction showed substantially lower activity (28.23 ± 1.91%). In *M. citrifolia*, the ethyl acetate fraction also demonstrated the highest ACE inhibitory activity (51.35 ± 0.94%), while the n-hexane and aqueous fractions exhibited lower activities of 33.26 ± 1.67% and 35.59 ± 5.30%, respectively. For *D. angustifolia*, the n-hexane fraction showed the greatest inhibitory activity (59.68 ± 3.30%), followed by the aqueous fraction (54.42 ± 0.82%) and the ethyl acetate fraction (45.81 ± 0.46%). Overall, substantial variation in ACE inhibitory activity was observed among the solvent fractions obtained from the three plant species.

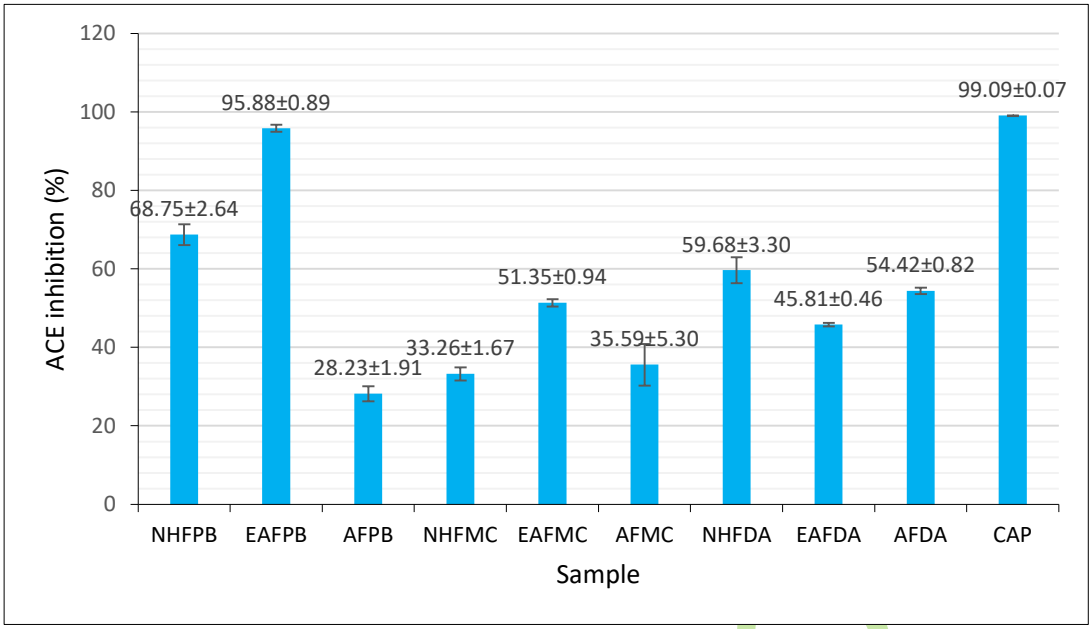

Figure 2. In vitro ACE inhibitory activity of solvent fractions obtained from *P. betle* leaves, *M. citrifolia* fruits, and *D. angustifolia* leaves at 100 µg/mL. Values are presented as mean ± SD (n = 3). Abbreviations: NHFPB = *Piper betle* leaf *n*-hexane fraction; EAFPB = *Piper betle* leaf ethyl acetate fraction; AFPB = *Piper betle* leaf aqueous fraction; NHFMC = *Morinda citrifolia* fruit *n*-hexane fraction; EAFMC = *Morinda citrifolia* fruit ethyl acetate fraction; AFMC = *Morinda citrifolia* fruit aqueous fraction; NHFDA = *Dracaena angustifolia* leaf *n*-hexane fraction; EAFDA = *Dracaena angustifolia* leaf ethyl acetate fraction; AFDA = *Dracaena angustifolia* leaf aqueous fraction; CAP = captopril.

4. DISCUSSION

The fraction yields obtained from the liquid–liquid fractionation of 1 g of ethanol extract from *P. betle* leaves, *M. citrifolia* fruits, and *D. angustifolia* leaves are presented in Table 1. As shown, the aqueous fractions consistently produced the highest yields (56.10–87.99%), whereas the ethyl acetate fractions yielded the lowest amounts (5.15–14.47%) across all plant species. This distribution indicates that a substantial proportion of the extracted constituents consisted of highly polar compounds that preferentially partitioned into the aqueous phase during fractionation. Polar phytochemicals, such as polysaccharides, glycosides, organic acids, amino acids, and certain phenolic compounds, are commonly enriched in aqueous fractions, resulting in greater recovery yields than less polar fractions [24,25]. Consequently, fraction yield primarily reflects the abundance and polarity of phytochemicals present in the crude extract rather than their biological activity.

Among the investigated plants, *M. citrifolia* fruit exhibited the highest aqueous fraction yield (87.99%), indicating a predominance of water-soluble metabolites. Previous phytochemical studies have reported that *M. citrifolia* fruits are rich in polysaccharides, iridoid glycosides, flavonoids, coumarins, and other polar constituents, which may explain the high recovery observed in the aqueous fraction [26]. *P. betle* and *D. angustifolia* are known to contain diverse phenolic compounds and flavonoids, while glycosides and saponins have also been reported in several extracts. Because these metabolites are generally moderately to highly polar, they tend to partition into polar solvent fractions during solvent fractionation [27,28]. Importantly, fraction yield was not directly associated with ACE inhibitory activity. Although aqueous fractions produced the highest yields, they did not necessarily exhibit the strongest ACE inhibitory effects. This finding suggests that biological activity depends on the enrichment of specific bioactive constituents rather than the total mass of recovered material, a phenomenon frequently observed in bioassay-guided fractionation studies.

Comparison of the three species revealed that the ethyl acetate fraction of *P. betle* exhibited the strongest ACE inhibitory activity (95.88%), suggesting enrichment of bioactive compounds with high affinity toward ACE. This activity was markedly higher than the previously reported ACE inhibitory activities of crude *P. betle* leaf extracts, which reached 56%, 48%, and 41% for the water, ethanol, and acetone extracts, respectively, using an HPLC-based ACE bioassay [29]. Such differences may be attributed to variations in sample preparation and analytical methodology, as the present study evaluated an ethyl acetate fraction using the ACE Kit–WST colorimetric assay, whereas the previous study employed crude extracts and an HPLC-based

assay. Statistical analysis further confirmed significant differences among the fractions. Although all datasets showed normal distribution according to the Shapiro–Wilk test ($p > 0.05$), variance homogeneity was not met (Levene's test, $p = 0.001$). Therefore, Welch ANOVA was applied and revealed significant differences in ACE inhibitory activity among the fractions ($p < 0.001$). Subsequent Games–Howell analysis showed that the ethyl acetate fraction of *P. betle* differed significantly from several less active fractions, including the aqueous fraction of *P. betle*, the n-hexane and aqueous fractions of *M. citrifolia*, and the n-hexane fraction of *D. angustifolia* ($p < 0.05$). Notably, no significant difference was observed between the ethyl acetate fraction of *P. betle* and captopril ($p = 0.103$), indicating a comparable inhibitory effect under the tested conditions.

The superior activity of the ethyl acetate fraction of *P. betle* may be associated with its abundance of hydroxychavicol and related phenolic constituents, which are recognized as major bioactive compounds with strong enzyme-inhibitory properties [30–32]. This finding is consistent with previous studies showing that ACE-inhibitory compounds are often concentrated in semi-polar fractions rich in phenolic compounds and flavonoids [33]. Likewise, several studies have reported that ethyl acetate fractions exhibit stronger ACE inhibitory activity than crude extracts or highly polar fractions because this solvent effectively concentrates semi-polar bioactive compounds, including phenolics and flavonoids, that can interact with the ACE active site or chelate the catalytic Zn^{2+} ion [33–36]. Despite its lower yield, the ethyl acetate fraction of *P. betle* exhibited substantially higher activity than the aqueous fraction, indicating effective enrichment of ACE-inhibitory metabolites during solvent partitioning.

Similarly, the ethyl acetate fraction of *M. citrifolia* showed the highest ACE inhibitory activity (51.35%), which may be attributed to flavonoids, scopoletin, rutin, and iridoid glycosides known for their antihypertensive properties [18,37]. In contrast, *D. angustifolia* exhibited the highest activity in the n-hexane fraction (59.68%), suggesting the involvement of relatively nonpolar constituents such as terpenoids and steroidal compounds. Collectively, these findings indicate that the ACE inhibitory activity of the studied plants is associated with different classes of phytochemicals distributed across fractions of varying polarity.

Overall, these results demonstrate that solvent polarity plays an important role in the enrichment of ACE-inhibitory compounds. Among the tested samples, the ethyl acetate fraction of *P. betle* exhibited the highest ACE inhibitory activity (95.88%) and showed no significant difference from captopril, highlighting its potential as a source of natural antihypertensive agents. Further bioassay-guided fractionation and phytochemical characterization are required to identify the compounds responsible for the observed activity.

5. CONCLUSION

Solvent fractionation influenced the enrichment of ACE-inhibitory compounds in *P. betle* L., *M. citrifolia* L., and *D. angustifolia* Roxb. Although the aqueous fractions produced the highest yields, the strongest ACE inhibitory activity was observed in the ethyl acetate fraction of *P. betle* (95.88%), which did not differ significantly from captopril. The highest ACE inhibitory activities of *M. citrifolia* and *D. angustifolia* were observed in the ethyl acetate and n-hexane fractions, respectively. These findings indicate that *P. betle*, particularly its ethyl acetate fraction, is a promising source of natural ACE inhibitors and warrants further phytochemical characterization and pharmacological evaluation.

Acknowledgements: The authors express their sincere appreciation to the Rector of Gunadarma University for the scholarship support and opportunity to pursue doctoral studies. The authors also acknowledge Universitas Pancasila and Universitas Nasional for their support through the Center for Botanicals and Chronic Diseases (CBCD) Indonesia, funded by the U.S. National Institutes of Health (NIH). In addition, the authors are grateful to the Research Center for Pharmaceutical Ingredients and Traditional Medicine, National Research and Innovation Agency (BRIN), Serpong, South Tangerang, Indonesia, for providing the laboratory facilities, resources, and technical assistance necessary for conducting this study.

Author contributions: Concept – A.K., E.S., M.H., S.A.; Design – A.K., E.S., M.H., P.D.N.L., S.A.; Supervision – S.A.; Resources – A.K., E.S., M.H., P.D.N.L., S.A.; Materials – A.K; Data Collection and/or Processing – A.K., E.S., M.H., S.A.; Analysis and/or Interpretation – A.K., E.S., M.H., P.D.N.L., S.A.; Literature Search – A.K., E.S., M.H., P.D.N.L., S.A.; Writing – A.K.; Critical Reviews – A.K., E.S., M.H., P.D.N.L., S.A.

Conflict of interest statement: The authors declared no conflict of interest.

REFERENCES

[1] S. et al. Oparil, "Hypertension," *Nat. Rev. Dis. Prim.*, vol. 22, no. 4, pp. 1–48, 2019, doi: 10.1038/nrdp.2018.14.Hypertension.

[2] H. Tomiyama, "Vascular function: a key player in hypertension," *Hypertens. Res.*, vol. 46, no. 9, pp. 2145–2158, 2023, doi: 10.1038/s41440-023-01354-3.

[3] L. L. Camargo, F. J. Rios, A. C. Montezano, and R. M. Touyz, "Reactive oxygen species in hypertension," *Nat. Rev. Cardiol.*, vol. 22, no. 1, pp. 20–37, 2025, doi: 10.1038/s41569-024-01062-6.

[4] T. J. Guzik, R. Nosalski, P. Maffia, and G. R. Drummond, "Immune and inflammatory mechanisms in hypertension," *Nat. Rev. Cardiol.*, vol. 21, no. 6, pp. 396–416, 2024, doi: 10.1038/s41569-023-00964-1.

[5] WHO, "Hypertension," 2025. https://www.who.int/news-room/fact-sheets/detail/hypertension (accessed Jul. 01, 2026).

[6] WHO, *Global report on hypertension 2025: High stakes – turning evidence into action*, vol. 406, no. 10517. Geneva, Switzerland: World Health Organization, 2025. doi: 10.1016/S0140-6736(25)02208-1.

[7] H. Triebel and H. Castrop, "The renin angiotensin aldosterone system," *Pflügers Arch. - J. Physiol.*, vol. 476, no. 5, pp. 705–713, 2024, doi: 10.1007/s00424-024-02908-1.

[8] K. E. Bernstein *et al.*, "A modern understanding of the traditional and nontraditional biological functions of angiotensin-converting enzyme," *Pharmacol. Rev.*, vol. 65, no. 1, pp. 1–46, 2013, doi: 10.1124/pr.112.006809.

[9] H. Ahmad, H. Khan, S. Haque, S. Ahmad, N. Srivastava, and A. Khan, "Review Article Angiotensin-Converting Enzyme and Hypertension: A Systemic Analysis of Various ACE Inhibitors, Their Side Effects, and Bioactive Peptides as a Putative Therapy for Hypertension," *J. Renin-Angiotensin-Aldosterone Syst.*, vol. 21, no. 2023, p. 7890188, 2023, doi: 10.1155/2023/7890188.

[10] P. K. Whelton *et al.*, "2017 ACC / AHA / AAPA / ABC / ACPM / AGS / APhA / ASH / ASPC / NMA / PCNA Guideline for the Prevention , Detection , Evaluation , and Management of High Blood Pressure in Adults : Executive Summary A Report of the American College of Cardiology / American," *Hypertension*, vol. 71, no. 6, pp. 1269–1324, 2018, doi: 10.1161/HYP.0000000000000066.

[11] S. Cutrell, I. S. Alhomoud, A. Mehta, A. H. Talasaz, and B. Van Tassell, "ACE - Inhibitors in Hypertension: A Historical Perspective and Current Insights," *Curr. Hypertens. Rep.*, vol. 25, no. 9, pp. 243–250, 2023, doi: 10.1007/s11906-023-01248-2.

[12] A. G. Atanasov *et al.*, "Natural products in drug discovery: advances and opportunities," *Nat. Rev. Drug Discov.*, vol. 20, no. 3, pp. 200–216, 2021, doi: 10.1038/s41573-020-00114-z.

[13] M. Madhumita, P. Guha, and A. Nag, "Bio-actives of betel leaf (Piper betle L.): A comprehensive review on extraction, isolation, characterization, and biological activity," *Phyther. Res.*, vol. 34, no. 10, pp. 2609–2627, 2020, doi: 10.1002/ptr.6715.

[14] N. N. Abrahim, M. S. Kanthimathi, and A. Abdul-Aziz, "Piper betle shows antioxidant activities, inhibits MCF-7 cell proliferation and increases activities of catalase and superoxide dismutase," *BMC Complement. Altern. Med.*, vol. 12, no. 220, pp. 1–11, 2012, doi: 10.1186/1472-6882-12-220.

[15] A. Kurniawan, E. Sinaga, M. Hanafi, P. Dewi Narrij Lotulung, and S. Abdillah, "Potential of bioactive compounds *Piper betle* L. as angiotensin converting enzyme inhibitors: molecular docking study and in silico analysis evaluation of physicochemical properties, drug-likeness, and toxicity," *J. Ilmu Kefarmasian Indones.*, vol. 24, no. 1, pp. 108–122, 2026, doi: 10.35814/jifi.v24i1.2083.

[16] C. H. Li *et al.*, *Morinda citrifolia L. (noni) and its potential in the management of systemic metabolic disorder (SMD)*, vol. 16, no. 24. Royal Society of Chemistry, 2025. doi: 10.1039/d5fo03892h.

[17] L. O. A. H. Hijriansyah, Hermilasari, H. Subair, Irianto, A. A. U. Armyn, and S. Hakim, "Study in vitro and in silico on effectiveness noni fruit extract (Morinda Citrifolia) to reducing hypertension," *Canrea J. Food Technol. Nutr. Culin. J.*, vol. 3, no. 2, pp. 57–64, 2020, doi: 10.20956/canrea.v3i2.329.

[18] R. T. Dewi *et al.*, "Quality control standardization of Indonesian noni fruit (Morinda citrifolia) extract and evaluation of their angiotensin-converting enzyme inhibitory activity," *Pharmacia*, vol. 69, no. 3, pp. 709–717, 2022, doi: 10.3897/pharmacia.69.e86854.

[19] A. K. Palu, R. A. Santiago, B. J. West, N. Kaluhiokalani, and J. Jensen, "The effects of Morinda citrifolia L. Noni on high blood pressure: A mechanistic investigation and case study," *ACS Symp. Ser.*, vol. 993, pp. 446–453, 2008, doi: 10.1021/bk-2008-0993.ch039.

[20] A. Triyanto *et al.*, "Ethnobotany of wild edible plants used by local communities in three districts along the upper Bengawan Solo River, Central Java, Indonesia," *Biodiversitas*, vol. 25, no. 4, pp. 1596–1605, 2024.

[21] B. Narender, N. Naveena, P. Pravalika, S. Kaleem, M. Vamshi, and J. R. Mandhadhi, "Pharmacological evaluation of root and leaf extracts of Dracaena reflexa var. angustifolia," *Innov. Pharm. Pharmacother.*, vol. 1, no. 1, pp. 141–146, 2017.

[22] L. H. Lam, T. Shimamura, S. Manabe, M. Ishiyama, and H. Ukeda, "Assay of angiotensin I-converting enzyme-inhibiting activity based on the detection of 3-hydroxybutyrate with water-soluble tetrazolium salt," *Anal. Sci.*, vol. 24, no. 8, pp. 1057–1060, 2008, doi: 10.2116/analsci.24.1057.

[23] L. H. Lam *et al.*, "Assay of angiotensin I-converting enzyme-inhibiting activity based on the detection of 3-hydroxybutyric acid," *Anal. Biochem.*, vol. 364, no. 2, pp. 104–111, 2007, doi: 10.1016/j.ab.2007.02.017.

[24] T. Lefebvre, E. Destandau, and E. Lesellier, "Selective extraction of bioactive compounds from plants using recent extraction techniques: A review," *J. Chromatogr. A*, vol. 1635, p. 461770, 2021, doi: 10.1016/j.chroma.2020.461770.

[25] N. A. Abd Aziz, R. Hasham, M. R. Sarmidi, S. H. Suhaimi, and M. K. H. Idris, "A review on extraction techniques and therapeutic value of polar bioactives from Asian medicinal herbs: Case study on Orthosiphon aristatus, Eurycoma longifolia and Andrographis paniculata," *Saudi Pharm. J.*, vol. 29, no. 2, pp. 143–165, 2021, doi: 10.1016/j.jsps.2020.12.016.

[26] S. Hou, D. Ma, S. Wu, Q. Hui, and Z. Hao, "Morinda citrifolia L .: A Comprehensive Review on Phytochemistry, Pharmacological Effects , and Antioxidant Potential," *Antioxidants*, vol. 14, no. 295, pp. 1–43, 2025.

[27] P. Biswas *et al.*, "Betelvine (Piper betle L.): A comprehensive insight into its ethnopharmacology , phytochemistry , and pharmacological , biomedical and therapeutic attributes," *J Cell Mol Med.*, vol. 26, pp. 3083–3119, 2022, doi: 10.1111/jcmm.17323.

[28] M. Xu, Y. J. Zhang, X. C. Li, M. R. Jacob, and C. R. Yang, "Steroidal saponins from fresh stems of Dracaena angustifolia," *J. Nat. Prod.*, vol. 73, no. 9, pp. 1524–1528, 2010, doi: 10.1021/np100351p.

[29] B. Somanadhan *et al.*, "An ethnopharmacological survey for potential angiotensin converting enzyme inhibitors from Indian medicinal plants," *J. Ethnopharmacol.*, vol. 65, pp. 103–112, 1999.

[30] N. A. Mohamad, A. A. Rahman, and S. H. S. A. Kadir, "Hydroxychavicol as a potential anticancer agent (Review)," *Oncol. Lett.*, vol. 25, no. 1, pp. 1–12, 2023, doi: 10.3892/ol.2022.13620.

[31] K. Murata *et al.*, "Hydroxychavicol: A potent xanthine oxidase inhibitor obtained from the leaves of betel, Piper betle," *J. Nat. Med.*, vol. 63, no. 3, pp. 355–359, 2009, doi: 10.1007/s11418-009-0331-y.

[32] N. Carsono, S. G. Tumilaar, D. Kurnia, D. Latipudin, and M. H. Satari, "A Review of Bioactive Compounds and Antioxidant Activity Properties of Piper Species," *Molecules*, vol. 27, no. 19, pp. 1–22, 2022, doi: 10.3390/molecules27196774.

[33] M. Y. Khan and V. Kumar, "Mechanism & inhibition kinetics of bioassay-guided fractions of Indian medicinal plants and foods as ACE inhibitors," *J. Tradit. Complement. Med.*, vol. 9, no. 1, pp. 73–84, 2019, doi: 10.1016/j.jtcme.2018.02.001.

[34] A. Ismail *et al.*, "Angiotensin Converting Enzyme (ACE) Inhibition Activity by Syzygium polyanthum Wight (Walp.) Leaves : Mechanism and Specificity," *Pharmacogn J.*, vol. 14, no. 1, pp. 76–84, 2022.

[35] A. R. Y. Eff, H. Z. Huri, M. Radji, A. Mun'im, F. D. Suyatna, and Y. Eden, "Angiotensin converting enzyme (ACE) inhibitors activity from purified compounds Fructus Phaleria macrocarpa (Scheff) Boerl," *BMC Complement. Med. Ther.*, vol. 23, no. 56, pp. 1–9, 2023.

[36] S. Ko *et al.*, "Angiotensin I-Converting Enzyme (ACE) Inhibition and Molecular Docking Study of Meroterpenoids Isolated from Brown Alga, Sargassum macrocarpum," *Int. J. Mol. Sci.*, vol. 24, no. 13, p. 11065, 2023.

[37] S. Zhou and G. Huang, "The chemical composition and pharmacological activities of Morinda citrifolia," *Appl. Biol. Chem.*, vol. 67, no. 104, pp. 1–18, 2024, doi: 10.1186/s13765-024-00960-0.